

Determination a Range of Analgesic Drugs Using Simple RP-HPLC Method with UV/VIS Detection

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Abstract

Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which temporarily affect, and in some instances completely eliminate.

Many different analytical methods had been done to determine some of analgesic by all analytical methods, such as spectrophotometer, electrochemical and chromatographic methods .

Various analytical chromatographic conditions were tested in this search by using HPLC-RP (UV-Vis), we have reached to the following separation conditions:

- 1- Sorbent : C18 Pyramid, 5 μ m
- 2- Mobile phase : (ACN + 0.1 %TFA)
(50 + 50) v/ v
- 3- ϕ = 1 ml/ min .
- 4- T= 25 °C
 λ_{\max} = 254 nm

By the proposed method , we achieved a sharp symmetric peak during :

Analgesics	t_R (min)
Paracetamol	2.0
Acetylsalicylic acid	2.5
Methyl 4- hydroxybenzoate	2.8
Ketoprofen	5.2
Flurbiprofen	10.5
Ibuprofen	13.2

$S = f(C)$ was applied in a various range depend on each analgesics, according to this concentration and liner equation we proceeded determining the quantity in each nutrition sample of the following : (Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen) and the Linear relationship was achieved in all studied analgesics , and it were different depending on the type of material studied, it was between (2-12)mg/L for(Paracetamol , Methyl 4- hydroxybenzoate, Ketoprofen, and Flurbiprofen), and (2-10)mg/L for Acetylsalicylic acid, and Ibuprofen , RSD=(0.0289- 0.2139)% . The proposed method was validated for specificity, linearity, accuracy, precision, and was successfully applied to pharmaceutical products.

Keywords: Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, Ibuprofen, RP-HPLC, and Analgesic

1-Introduction

This present paper describes a sensitive and simple RP-HPLC method with UV/VIS detection for determination a number of analgesics in one sample .

Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which temporarily affect, and in some instances completely eliminate, sensation. Analgesics include paracetamol (known in North America as acetaminophen or simply APAP), the nonsteroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioiddrugs such as morphine and oxycodone.

Analgesics are those drugs that mainly provide pain relief. The primary classes of analgesics are the narcotics, includingadditional agents that are chemically based on the morphine molecule but have minimal abuse potential; nonsteroidal anti-inflammatory drugs (NSAIDs) including the salicylates; and acetaminophen. Other drugs, notably the tricyclicantidepressants and antiepileptic agents such as gabapentin, have been used to relieve pain, particularly neurologicpain, but are not routinely classified as analgesics. Analgesics provide symptomatic relief, but have no effect on thecause, although clearly the NSAIDs, by virtue of their dual activity, may be beneficial in both regards

1-1- Paracetamol $C_8H_9NO_2$

Odorless white crystalline solid. Bitter taste. pH (saturated aqueous solution) about 6

Paracetamol, also known as acetaminophen or APAP, is a medication used to treat painand fever[1] .

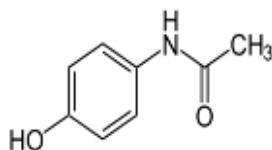


Figure (1): structure of Paracetamol

Paracetamol consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para (1,4) pattern.[2] The amide group is acetamide (ethanamide). It is an extensively conjugated system, as the lone pair on the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair, the p orbital on the carbonyl carbon, and the lone pair on the carbonyl oxygen are all conjugated. The presence of two activating groups also make the benzene ring highly reactive toward electrophilic aromatic substitution. As the substituents are ortho, para-directing and para with respect to each other, all positions on the ring are more or less equally activated. The conjugation also greatly reduces the basicity of the oxygens and the nitrogen, while making the hydroxyl acidic through delocalisation of charge developed on the phenoxide anion. Paracetamol is part of the class of drugs known as "aniline analgesics"; it is the only such drug still in use today.[3] It is not considered an NSAID because it does not exhibit significant anti-inflammatory activity (it is a weak COX inhibitor).[4-5] This is despite the evidence that paracetamol and NSAIDs have some similar pharmacological activity[6].

1-2- Acetylsalicylic acid $C_9H_8O_4$

also known as Aspirin. Odorless white crystals or crystalline powder with a slightly bitter taste. Aspirin decomposes rapidly in solutions of ammonium acetate or the acetates, carbonates, citrates, or hydroxides of the alkali metals. It is stable in dry air, but gradually hydrolyses in contact with moisture to acetic and salicylic acids. In solution with alkalis, the hydrolysis proceeds rapidly and the clear solutions formed may consist entirely of acetate and salicylate[7]. Like flour mills, factories that make aspirin tablets must pay attention to how much of the powder gets into the air inside the building, because the powder-air mixture can be explosive.

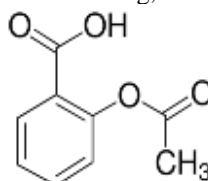


Figure (2): structure of Acetylsalicylic acid

The National Institute for Occupational Safety and Health (NIOSH) has set a recommended exposure limit in the United States of 5 mg/m³ (time-weighted average).[8]. In 1989, the Occupational Safety and Health Administration (OSHA) set a legal permissible exposure limit for aspirin of 5 mg/m³, but this was vacated by the AFL-CIO v. OSHA decision in 1993[9].

1-3- Methyl 4- hydroxybenzoate $C_8H_8O_3$

also known as Methyl paraben; Methyl p-hydroxybenzoate

Almost odourless, small colourless crystals or white crystalline powder Methylparaben serves as a pheromone for a variety of insects and is a component of queen mandibular pheromone. Some plants produce methylparaben, example thale cress[10]. It is commonly used in the preparation of liquid dosage forms. There is controversy about whether methylparaben or propylparabens are harmful at concentrations typically used in body care or cosmetics. Methylparaben and propylparaben are considered generally recognized as safe (GRAS) by the USFDA for food and cosmetic antibacterial

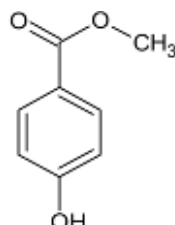


Figure (3): structure of Methyl 4- hydroxybenzoate

preservation[11]. Methylparaben is readily metabolized by common soil bacteria, making it completely biodegradable. Methylparaben is readily absorbed from the gastrointestinal tract or through the skin. It is hydrolyzed to p-hydroxybenzoic acid and rapidly excreted in urine without accumulating in the body.[6] Acute toxicity studies have shown that methylparaben is practically non-toxic by both oral and parenteral administration in animals. In a population with normal skin, methylparaben is practically non-irritating and non-

sensitizing; however, allergic reactions to ingested parabens have been reported.[12] Studies indicate that methylparaben applied on the skin may react with UVB, leading to increased skin aging and DNA damage [13-14].

1-4- Ketoprofen $C_{16}H_{14}O_3$

It is one of the propionic acid class of nonsteroidal anti-inflammatory drugs (NSAID) with analgesic and antipyretic effects[15].

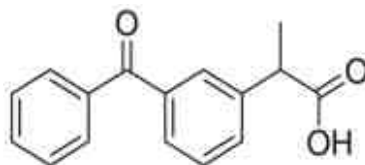


Figure (4): structure of Ketoprofen

1-5- Flurbiprofen $C_{15}H_{13}FO_2$

Flurbiprofen is a member of the phenylalkanoic acid derivative family of nonsteroidal anti-inflammatory drugs (NSAIDs). It is primarily indicated as a pre-operative anti-miotic (in an ophthalmic solution) as well as orally for arthritis or dental pain. Side effects are analogous to those of ibuprofen[16].

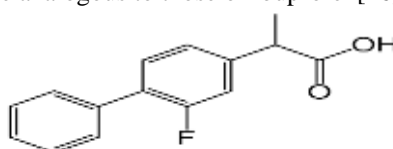


Figure (5): structure of Flurbiprofen

Flurbiprofen is a nonsteroidal antiinflammatory drug (NSAID) used in treatment of mild-to-moderate pain and symptoms of chronic arthritis. Flurbiprofen has been linked to a low rate of serum enzyme elevations during therapy and to rare instances of clinically apparent acute liver injury[17].

1-6-Ibuprofen $C_{13}H_{18}O_2$

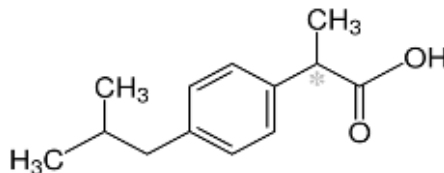


Figure (6): structure of Ibuprofen

Ibuprofen is practically insoluble in water, but very soluble in most organic solvents like ethanol, methanol, acetone and dichloromethane[18]. The original synthesis of ibuprofen by the Boots Group started with the compound 2-methylpropylbenzene. The synthesis took six steps. A modern, greener technique for the synthesis involves only three steps[19].

- there are many analytical methods have been reported for determination: Paracetamol [20-23]; Acetylsalicylic acid [24-26]; Methyl 4- hydroxybenzoate [27-29] ; Ketoprofen [30-33]; Flurbiprofen[34-37]; and Ibuprofen[38-42]. in its samples.
- This present paper describes a sensitive and simple RP-HPLC method with UV/VIS detection for determination of (Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen) all together in one sample, due to the intensity of their production by pharmaceutical laboratories for the urgent need for these drugs as analgesics.

2-Result and discussion:

2-1 Analytical conditions :

In this search we fix on chromatographic methods because it's our goal, (HPLC-UV) method was applied on a column C18 Pyramid, 5 μ m to analyses. RP-HPLC method was used for measurement of the concentration of (Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen), standards for each substances are prepared by accurately weighing 25 mg of analgesics powder and adding 10 to 20 ml of DI water to make stock solutions of 1.0 mg/ml for each.

Table (1) : The optimum chromatographic conditions we have achieved were :

- 1- Sorbent : C18 Pyramid, 5 μ m
- 2- Mobile phase : (ACN + 0.1 %TFA)
(50 + 50) v/ v
- 3- ϕ = 1 ml/ min .
- 4- T= 25 $^{\circ}$ C
- 5- λ_{\max} = 254 nm

ACN : Acetonitrile , TFA : trimethylamine

By using above conditions , the peak of Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen , show in figures (7, 8, 9, 10,

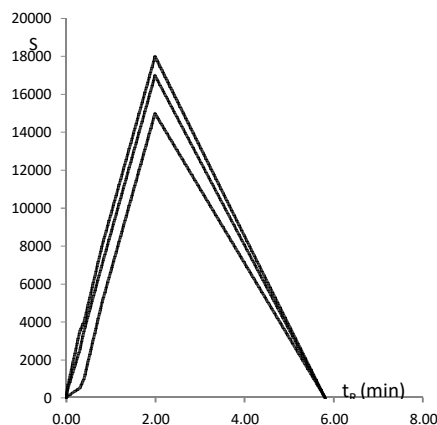


Figure (7): Chromatographic peak of Paracetamol , Sorbent : C18 Pyramid, 5 μ m , Mobile phase (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm .

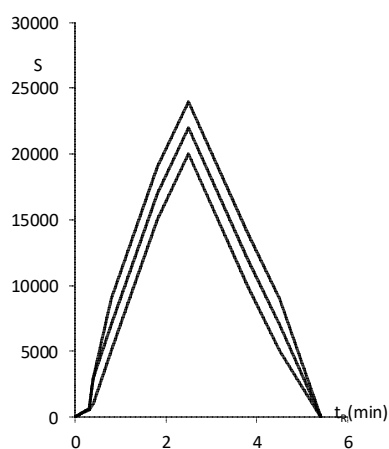


Figure (8): Chromatographic peak of Acetylsalicylic acid Sorbent : C18 Pyramid, 5 μ m , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm .

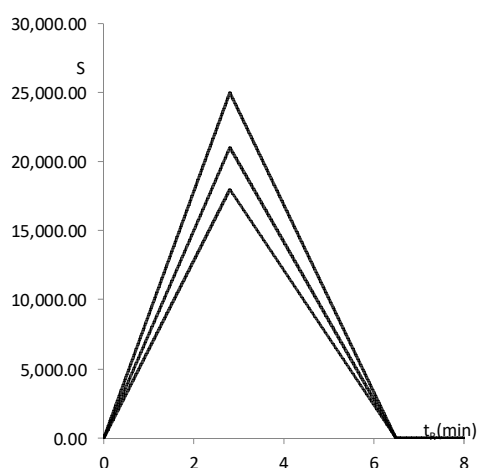


Figure (9): Chromatographic peak of Methyl 4- hydroxybenzoate Sorbent : C18 Pyramid, 5 μ m , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm . 11,and 12). From the figures, we found that the t_R of each studied analgesics are separate, table (2).

Table (2) : the amount of t_R for the studied analgesics in separate conditions as: C18 Pyramid; Mobile phase : (ACN + 0.1 %TFA) (50+50)v/v ; ϕ = 1 ml/ min ; λ_{\max} = 254 nm .

Analgesics	t_R (min)
Paracetamol	2.0
Acetylsalicylic acid	2.5
Methyl 4- hydroxybenzoate	2.8
Ketoprofen	5.2
Flurbiprofen	10.5
Ibuprofen	13.2

We can see From the figures. (7, 8, 9, 10, 11,and 12). That we can separate the common solution of Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen by using the optimum conditions , summarized in table (1) , that is the target of our search .

2-2 Study of slandered solutions, $S = f(C)$:

Five common standers solution of Paracetamol, Acetylsalicylic acid, Methyl 4- hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen were prepared and injected on C18 Pyramid, 5 μ m column and the analysis carried out by elution with (ACN + 0.1 %TFA) , (50+50) v/ v , λ_{\max} = 254 nm , The liner relation between peak surface and the Analgesics concentration , we achieved in the wide range, as show in figures (13,14, 15, 16, 17, and 18).

2-3-preparation of experimental Analgesics samples :

To be sure about the accuracy and precision of our proposed chromatographic method , the proposed method was applied on experimental common solution Analgesics samples, for that 3 slandered solutions were prepared, their concentrations include in the linear rang which we obtained above, and each concentration was repeated 3 times, then we have done some statistic study, table (3).

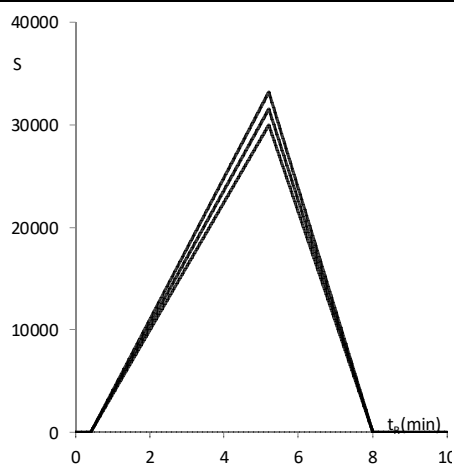


Figure (10): Chromatographic peak of Ketoprofen Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , $\phi = 1 \text{ ml/ min}$, $\lambda_{\text{max}} = 254 \text{ nm}$.

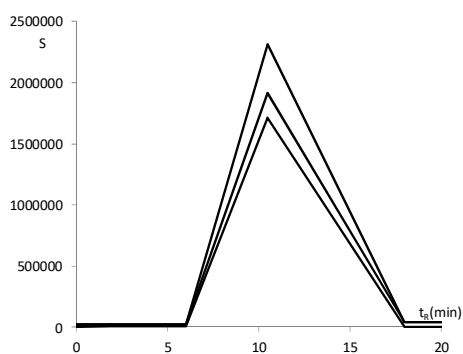


Figure (11): Chromatographic peak of Flurbiprofen Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , $\phi = 1 \text{ ml/ min}$, $\lambda_{\text{max}} = 254 \text{ nm}$

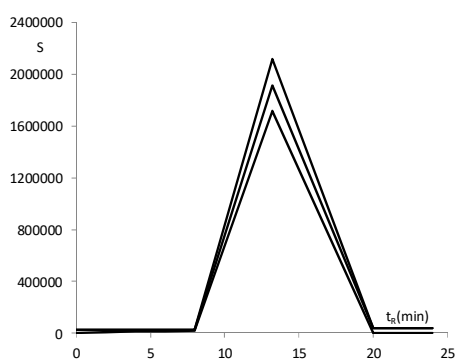


Figure (12): Chromatographic peak of Ibuprofen Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , $\phi = 1 \text{ ml/ min}$, $\lambda_{\text{max}} = 254 \text{ nm}$

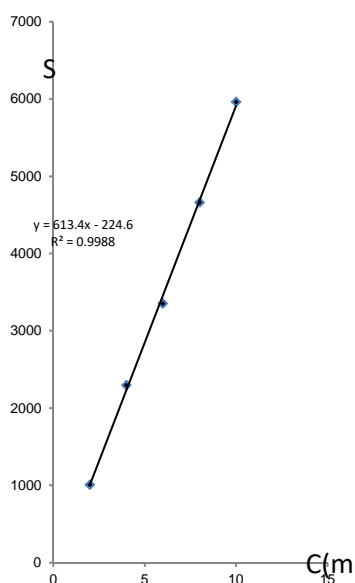


Figure (13) : the linear range between surface peaks and Paracetamol concentration ,in separate conditions as : Sorbent : C18 Pyramid, 5 μ m , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm

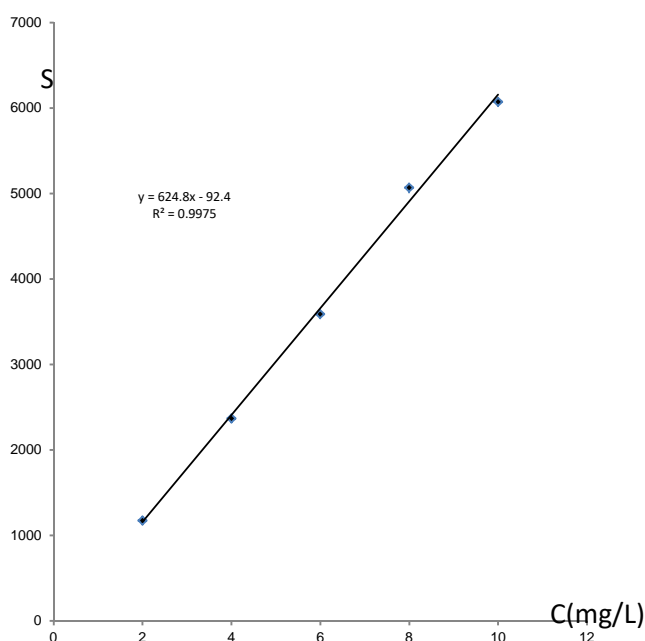


Figure (14) : the linear range between surface peaks and Acetylsalicylic acid concentration ,in separate conditions as : Sorbent : C18 Pyramid, 5 μ m , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm

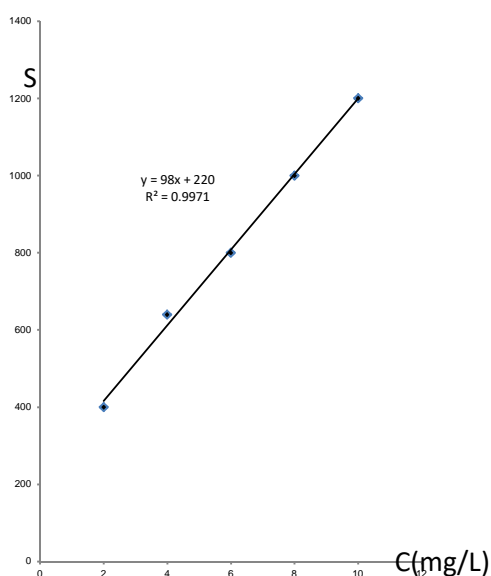


Figure (15) : the linear range between surface peaks and Methyl 4- hydroxybenzoate concentration in separate conditions as : Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{max} = 254 nm

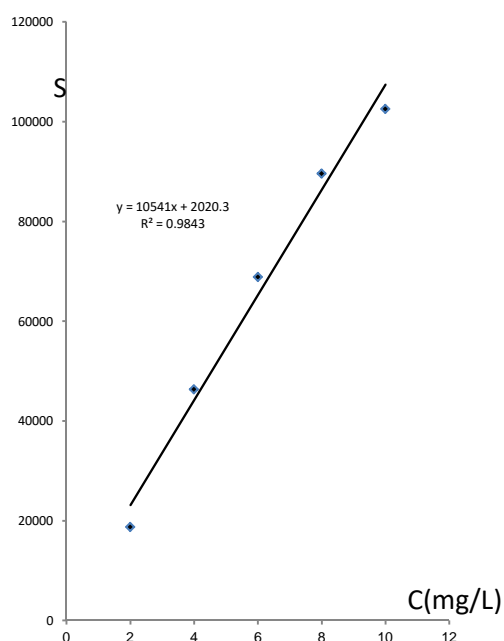


Figure (16) : the linear range between surface peaks and Ketoprofen concentration in separate conditions as : Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{max} = 254 nm

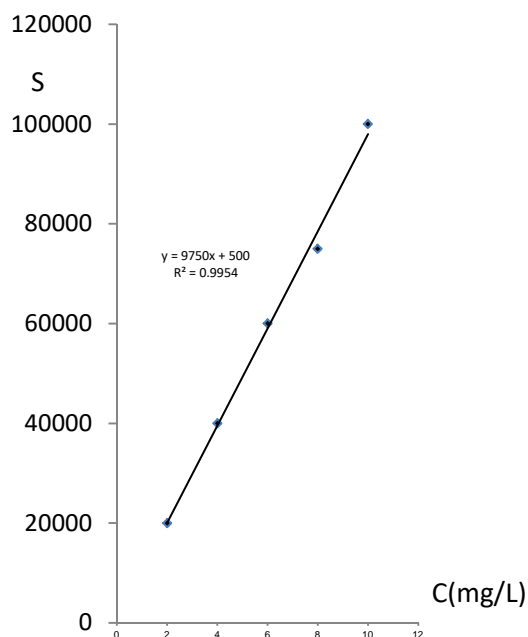


Figure (17) : the linear range between surface peaks and Flurbiprofen Concentration in separate conditions as :
 Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 % TFA)
 (50 +50) v/ v , $\phi = 1 \text{ ml/ min}$, $\lambda_{\text{max}} = 254 \text{ nm}$

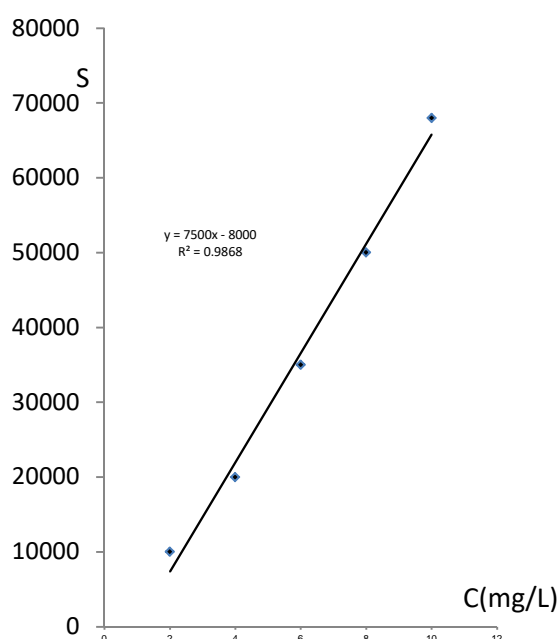


Figure (18) : the linear range between surface peaks and Ibuprofen Concentration in separate conditions as :
 Sorbent : C18 Pyramid, 5 μm , Mobile phase : (ACN + 0.1 % TFA)
 (50 +50) v/ v , $\phi = 1 \text{ ml/ min}$, $\lambda_{\text{max}} = 254 \text{ nm}$

Table(3) : determination experimental analgesics (Paracetamol, Acetylsalicylic acid, Methyl 4-hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen) samples using HPLC-RP, Sorbent : C18 Pyramid, 5 μ m, Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm, (n=3 , α =0.95) .

Studied analgesics	Taken concentration mg /L	Found concentration $\bar{X} \pm \Delta X$ mg/ L	RSD%	Recovery %
Paracetamol	3	3.01 \pm 0.028	0.7413	102.7
	7	6.79 \pm 0.016	1.3600	96.3
	9	10.03 \pm 0.016	0.9560	100.7
Acetylsalicylic acid	3	2.81 \pm 0.028	0.9613	100.2
	7	7.32 \pm 0.016	1.3600	98.89
	9	9.02 \pm 0.016	0.9560	100.5
Methyl 4-hydroxybenzoate	3	3.21 \pm 0.075	1.0413	99.7
	7	7.29 \pm 0.018	1.3600	100.3
	9	9.03 \pm 0.016	0.9560	100.6
Ketoprofen	3	2.99 \pm 0.045	0.4413	99.7
	7	7.02 \pm 0.013	0.2401	101.3
	9	8.99 \pm 0.015	0.9860	100.2
Flurbiprofen	3	3.00 \pm 0.045	0.4413	95.7
	7	7.01 \pm 0.013	0.2401	101.3
	9	9.09 \pm 0.015	0.9860	100.2
Ibuprofen	3	3.08 \pm 0.032	0.4413	99.8
	7	6.99 \pm 0.012	0.2401	103.7
	9	8.99 \pm 0.017	0.9860	101.2

2-4-Natural samples :

- The proposed method was applied in natural samples, taken from the Pharmacies and Drug Stores
- 1- For tablet : crashed 10 tablets and mixer until a homogeneous crushed has been achieved
 For Syrup : mix the 4 contents bottles until a homogeneous crushed has been achieved
 - 2- Added to the homogeneous crushed sample or to the syrup solution deionized water , stir on ultra sound for 30 min until completely dissolved solution achieved,
 - 3- isolated by two steps: first with ash less paper , second by special filter for HPLC .
 - 4- Diluted to 100 ml by water for HPLC [these solution is : mother sample solution]
- Each mother sample solution was diluted according to analgesics concentration by recording analytical signal (peak analgesics surface) at same time retention time for each analgesics (t_R), calculation the analgesics concentration in each sample was applied parallel by standard solution at same time, result in table(4) .

Table(4) : determination experimental analgesics (Paracetamol, Acetylsalicylic acid, Methyl 4-hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen) samples using HPLC- RP , Sorbent : C18 Pyramid, 5 μ m , Mobile phase : (ACN + 0.1 %TFA) (50 +50) v/ v , ϕ = 1 ml/ min , λ_{\max} = 254 nm, (n=3 , α =0.95) .

Analgesics	Sample	Found concentration $\bar{X} \pm \Delta\bar{X}$ mg/100 gr	RSD%
Paracetamol	Tablet	20.013 \pm 0.016	0.1413
		20.340 \pm 0.017	0.0289
		23.240 \pm 0.119	0.0613
	Syrup	15.913 \pm 0.114	0.0413
		16.813 \pm 0.1632	0.1159
		18.918 \pm 0.7265	0.1559
Acetylsalicylic acid	Tablet	30.513 \pm 0.1187	0.2139
		25.018 \pm 0.0194	0.1143
		28.713 \pm 0.1563	0.1716
Methyl 4- hydroxybenzoate	Tablet	21.017 \pm 0.0152	0.1359
		26.147 \pm 0.1043	0.1012
		23.240 \pm 0.1196	0.1543
Ketoprofen	Tablet	24.026 \pm 0.0058	0.1723
		26.537 \pm 0.1409	0.1275
		23.944 \pm 0.1276	0.1016
Flurbiprofen	Tablet	30.526 \pm 0.1784	0.1423
		26.997 \pm 0.1033	0.1389
		23.944 \pm 0.0276	0.1423
Ibuprofen	Tablet	28.428 \pm 0.1184	0.0983
		26.999 \pm 0.1070	0.1653
		30.344 \pm 0.1293	0.1334
	Syrup	33.828 \pm 0.1277	0.0545
		28.994 \pm 0.1072	0.1893
		30.947 \pm 0.1431	0.1479

From the table we concluded: a rapid and sensitive high performed liquid chromatography proposed method was carried out, accordingly we can determined analgesics (Paracetamol, Acetylsalicylic acid, Methyl 4-hydroxybenzoate, Ketoprofen, Flurbiprofen, and Ibuprofen)

in the wide range of samples , the method has a repeatedly and accuracy, we can observe from the lower RSD; and a high precision which can observed from the recovery, Accuracy is the degree of agreement between test results and true values. The precision of this method is the degree of agreement among individual test results when an analysis is applied repeatedly to multiple samplings. Precision is measured by injecting a series of standards and then calculating the relative standard deviation of retention times and areas or peak heights. Precision may be measured at three levels: repeatability, intermediate precision, and reproducibility.. Repeatability off low rates, gradient formation, and injection volumes can affect precision, as can response stability of the detector, aging of the column, and temperature stability of the column oven. The equipment should be inspected on a regular basis using the test methods recommended by the supplier to ensure reliability, high performance, and good analytical results.

Reference

- 1- The American Society of Health-System Pharmacists. from the original on 5 June 2016.
- 2- Bales, JR; Nicholson JK; Sadler PJ (1985). Two-dimensional proton nuclear magnetic resonance maps of acetaminophen metabolites in human urine. Clinical Chemistry. 31 ,5. 757–762.
- 3- Bertolini A; Ferrari A; Ottani A; Guerzoni S; Tacchi R; Leone S, (2006). Paracetamol new vistas of an old drug. CNS Drug Reviews. 12, 3–4, 250–75.

- 4- Viswanathan, A. N.; Feskanich, D.; Schernhammer, E. S., Hankinson, S. E. (2008). Aspirin, NSAID, and Acetaminophen Use and the Risk of Endometrial Cancer. *Cancer Research*. 68 ,7, p. 2507–13
- 5- Altinoz, M. A., Korkmaz, R. (2004). NF-kappaB, macrophage migration inhibitory factor and cyclooxygenase-inhibitions as likely mechanisms behind the acetaminophen- and NSAID-prevention of the ovarian cancer. *Neoplasma*. 51 ,4,p. 239–247.
- 6- Byrant, Bronwen; Knights, Katleen; Salerno, Evelyn (2007). *Pharmacology for health professionals*. Elsevier. p. 270.
- 7- Reynolds, E.F., ed. (1982). Aspirin and similar analgesic and anti-inflammatory agents. Martindale: the extra pharmacopoeia (28th ed.). Rittenhouse Book Distributors. p. 234–82.
- 8- Acetylsalicylic acid. Pocket Guide to chemical hazards. U.S. National Institute for Occupational Safety and Health. 13 February 2015.
- 9- Appendix G, 1989 Air contaminants update project – Exposure limits NOT in effect. NIOSH pocket guide to chemical hazards. National Institute for Occupational Safety and Health. 13 February 2015.
- 10- Walker TS, Bais HP; Halligan KM; Stermitz FR, Vivanco JM (2003). *Metabolic profiling of root exudates of Arabidopsis thaliana*. *Journal of Agricultural and Food Chemistry*. 51 ,9,.2548–54.
- 11- Parabens, Food and Drug Administration
- 12- Soni MG; Taylor SL;Greenberg NA, Burdock GA (2002). Evaluation of the health aspects of methyl paraben: a review of the published literature. *Food and Chemical Toxicology*. 40 ,10,p. 1335–73.
- 13- Handa, O; Kokura, S; Adachi, S; Takagi, T; Naito, Y; Tanigawa, T; Yoshida, N, Yoshikawa, T ,2006, Methylparaben potentiates UV-induced damage of skin keratinocytes. *Toxicology*. 227 ,1– 2, 62–72.
- 14- Jump up Okamoto; Yoshinori; Hayashi;Tomohiro; Matsunami, Shinpei; Ueda, Koji; Kojima Nakao (2008), Combined Activation of Methyl Paraben by Light Irradiation and Esterase Metabolism toward Oxidative DNA Damage. *Chemical Research in Toxicology*. 21 ,8,p. 1594–9.
- 15- Kantor, T. G. (1986), *Ketoprofen: a review of its pharmacologic and clinical properties*. *Pharmacotherapy*. 6 ,3,p. 93–103.
- 16- Lexicomp. Wolters Kluwer. Retrieved 25 September 2015.
- 17- Drug BankRecord Name: Flurbiprofen URL: <http://www.drugbank.ca/drugs/DB00712>
- 18- Brayfield, A, ed. (14 January 2014). Ibuprofen. Martindale: The Complete Drug Reference. London, UK: Pharmaceutical Press.
- 19- The Synthesis of Ibuprofen. Royal Society of Chemistry. Archived from the original on 18 April 2016.
- 20- Luna AS, Pinho JSA, 2014, Determination of Paracetamol and Ibuprofen in Tablets and Urine Using Spectrofluorimetric Determination Coupled with Chemometric Tools. *Austin J Anal Pharm Chem*. 1,1,p.1001.
- 21- Buddha Ratna Shrestha, Raja Ram Pradhananga,2009, Spectrophotometric Method for the Determination of Paracetamol , J. Nepal Chem. Soc., vol. 24.
- 22- Mahesh Attimarad, Simultaneous 2011,determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation, *Pharm Methods*. Jan-Mar; 2,1, 61–66.
- 23- Alaa El-Gindy ,Khalid Abdel-Salam Attia,Mohammad Wafaa Nassar,Hamed Hamed Abu Seada ,Maisra Al-Shabrawi Shoeib 2013,HPLC method for determination of paracetamol, psedoeohedrine, triprolidine, methylparaben, propylparaaben, sodium benzoate, and their related substances in pharamaceutical syrup, *J.liquid chromatography and related technologies*, v.36, 9.
- 24- MaryJean Sawyer, Vimal Kumar, 2003,A Rapid High-Performance Liquid Chromatographic Method for the Simultaneous Quantitation of Aspirin, Salicylic Acid, and Caffeine in Effervescent Tablets, *Journal of Chromatographic Science*, Vol. 41.
- 25- Franeta JT.; Agbaba D.; Eric S.; Pavkov S.; Aleksic M,Vladimirov S.2002, HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets. *Farmaco. Sep*;57,9, 709-13.
- 26- José Luiz Neves de Aguiar; Katia Christina Leandro; Shirley de Mello Pereira Abrantes, André Luis Mazzei Albert,2009, Development of a new analytical method for determination of acetylsalicylic and salicylic acids in tablets by reversed phase liquid chromatography, *Brazilian Journal of Pharmaceutical Sciences* vol. 45, N. 4.
- 27- G. A. Shabir, 2010, A New Validated HPLC Method for the Simultaneous Determination of 2-phenoxyethanol, Methylparaben, Ethylparaben and Propylparaben in a Pharmaceutical Gel, *Indian J Pharm Sci*. 72,4, 421–425.
- 28- Ghulam A. Shabir, 2011, Simultaneous analysis of phenothrin, methyl-4-hydroxybenzoate and propyl-4-hydroxybenzoate in human head lice medicin e by HPLC, *j.liquid Chromatography and related technologies* ,v.34, 16.
- 29- Rajesh M. Kashid; Santosh G. Singh, Shrawan Singh,2011,Simultaneous Determination of Preservatives (Methyl Paraben and Propyl Paraben) in Sucralfate Suspension Using High Performance Liquid Chromatography, *E-Journal of Chemistry*, 8,1, 340-346.

- 30-Allegrini A1; Nuzzo L.; Zucchelli M.; Scaringi AT.; Felaco S.; Giangreco D.; Pavone D.; Toniato E.; Mezzetti A.; Martinotti S.; Comuzio S., Di Grigoli M.,2009, Fast HPLC method for the determination of ketoprofen in human plasma using a monolithic column and its application to a comparative bioavailability study in man. *Arzneimittelforschung*. 59,3,P.135-40.
- 31-Farya Zafar1; Muhammad Harris Shoaib1; Asia Naz; Rabia Ismail Yousuf, Huma Ali, 2013,Determination of Ketoprofen in Human Plasma by RP-HPLC, *American Journal of Analytical Chemistry*, 4, P.252-257.
- 32-J..Negru;D.Saveta;L.;Vlase,D.Iacob;M.Achim;V.Dorneanu,2015, High throughput HPLC method for rapid quantification of ketoprofen in human plasma, *FARMACIA*, Vol. 63, 5.
- 33-YadollahAzarmi'HadiValizadeh' Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation,*J. pharmaceutical andBiomedical Analysis*, v. 39,p.624-630.
- 34-Muhammad Akhlaq; Gul Majid Khan; Abdul Wahab;Arshad Khan; Abid Hussain; Asif Nawaz, Hamdy Abdelkader,2011, A simple high-performance liquid chromatographic practical approach for determination of flurbiprofen, *J Adv Pharm Technol* ,Jul-Sep; 2,3,p. 151–155.
- 35- Bilal Yilmaz1, Ali Fuat Erdem, 2015,Determination of Flurbiprofen in Human Plasma by High-Performance Liquid Chromatography, *Journal of Chromatographic Science* ,53,1443–1448.
- 36-Nagwa H. Foda ; O. M. Al Gohary,1994, High Performance Liquid Chromatographic Determination of Flurbiprofen in Pharmaceutical Dosage Forms, *Analytical Letters* V. 27, N.13.
- 37- K. S. Albert; W. R. Gillespie, A. Raabe, M. Garry,1984, Determination of flurbiprofen in human serum by reverse-phase high-performance liquid chromatography with fluorescence detection, *Journal of pharmaceutical sciences*, v.73.issue12, p. 1823–1825.
- 38-Sylvester Okhuelegbe;EragMathew; IkhuoriaArhewoh;Rosemary Ngozi;Chibuogwu,Magnus Amaralwuagwu,2015, A comparative UV–HPLC analysis of ten brands of ibuprofen tablets, *Asian Pacific Journal of Tropical Biomedicine*, V. 5, No. 10, P. 880-884.
- 39-Md. Sarowar; Jahan, Md;Jahirul Islam; Rehana Begum; Ruhul Kayesh, Asma Rahman'2014' A Study of Method DevelopmentValidation and Forced Degradation for Simultaneous Quantification of Paracetamol and Ibuprofen in Pharmaceutical Dosage Form by RP-HPLC Method, *Anal Chem Insights*, 9: 75–81.
- 40- Selvadurai Muralidharan , Subramania Nainar Meyyanathan,2011, Development and Validation of a HPLC and an UV Spectrophotometric Methods for Determination of Dexibuprofen in Pharmaceutical Preparations, *ISRN Pharm.* v.2011.
- 41- Pinak M. Sanchaniya; Falgun A. Mehta, Nirav B. Uchadadiya, 2013,Development and Validation of an RP-HPLC Method for Estimation of Chlorpheniramine Maleate, Ibuprofen, and Phenylephrine Hydrochloride in Combined Pharmaceutical Dosage Form, *Chromatography Research International*, Volume 2013 ,v. 2013.
- 42- M. Alsirawani; M.Amin; B.Alkasmil; K. Alhareth,El-hammadi, 2013,development and validation of a simple HPLC method for the determination of ibuprofen sticking onto punch faces, *International Journal of Pharmacy and Pharmaceutical Sciences*, V.5, p.1491.